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The Induction of Intestinal Neoplasms in Rats with the Glycoside Cycasin and its Aglycone*

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With 8 Figures in the Text

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An investigation was started at the National Institutes of Health in 1961 into the question whether the seed of *Cycas circinalis* L., indigenous to Guam, possessed a neurotoxin which might explain the high frequency of certain neurologic diseases on that island. Interest in this particular plant came about through personal observations by Whiting who had studied the use of the plant as a source of food by the inhabitants and her subsequent familiarity with the literature on its toxic properties. The reader is referred to her review on toxicity of cycads which covers the period up to 1963 (Whiting).

While searching for evidence of neurotoxic effects in rats fed dried and ground seed of *Cycas circinalis* from Guam, it was noted that such material possessed marked hepatotoxic properties and that the degree of liver injury paralleled its concentration in the diet. The nature of the acute and subacute alterations in the liver suggested, furthermore, the possibility that the toxin in the crude material might be carcinogenic as well. This was confirmed in preliminary experiments in rats permitted to live sufficiently long for neoplasms to develop. The results of these early studies showed that neoplasms occurred predominantly in liver and kidneys in the majority of the rats which had been fed the crude cycad seed material over long periods of time [Laqueur et al. (1)].

Further search into the chemistry of cycads revealed that glycosides had been isolated from various members of the family of "cycadaceae" which were azoxy-glycosides differing only in the sugar component. In *cycasin*, the glycoside isolated from *Cycas circinalis* and *Cycas revoluta*, the sugar was glucose, its chemical formula being $\beta$-D-glucosyl-oxyazoxy methane [Nishida et al. (1), Riggs].

Subsequent studies showed that both the acute and long-term effects of the crude material could be reproduced as readily with the crystalline cycasin, suggesting that the effects of the crude material were essentially due to its content of cycasin [Laqueur (2)].

Further experiments in rats with the unstable aglycone of cycasin, methylazoxy methanol (MAM), isolated from cycad seed showed that it produced liver tumors and hyperplastic lesions in renal tubules (Matsumoto and Strong). Earlier studies revealed that the aglycone produced hepatotoxic effects even after intraperitoneal administration, a route which was found ineffective in the case of cycasin [Nishida et al. (2), Kobayashi and Matsumoto (2)].

* Dedicated to Professor Dr. Dr. h. c. Carl Krauspe on the occasion of his 70th birthday.
The aglycone acetate recently synthesized by MATSUMOTO et al. (2) is considerably more stable than the aglycone itself and is presently being tested for its carcinogenic activity in germfree rats using various routes of administration.

While the majority of toxic, biologic and carcinogenic effects of cycasin or the aglycone were similar to those described with dimethylnitrosamine (DMN) [MAGEE and BARNES (1, 2), MAGEE and SCHONTAL (3)], there was one notable exception in that neoplasms of the intestines developed with cycad materials but not with DMN. It is the purpose of this report to summarize our observations on intestinal neoplasms in rats fed the crude cycad material or cycasin and in rats given intraperitoneal injections of the aglycone. The discussion concerns the possible significance of these observations in relation to cycasin metabolism and future studies.

Material and Methods

The rats used in the majority of experiments were of the Osborne-Mendel (OM) and Sprague-Dawley (SD) strains maintained at NIH and obtained at weanling age. They were fed the basal diet until 30 days old when they were started on the experimental diets. Two groups were started on the experiment at 54 and 90 days respectively, to examine the effect of onset and established sexual maturity on the development of tumors. The pertinent data covering age, dose levels and duration of exposure are given in the accompanying Table 1.

Cycad meal (the crude ground and dried seed of Cycas circinalis) was prepared at the Institute from fresh seeds shipped from Guam. The dried material contained 2.3 gm of cycasin per 100 gm and was fed to the rats at a concentration of 1.0, 1.5 and 2.5 percent respectively.

Cycasin, isolated in crystalline form by the Department of Biochemistry of Kagoshima University, Kagoshima, Japan, was mixed with the basal diet at concentrations of 20, 40, 60, 100 and 400 mg per 100 gm diet. The rats chronically exposed to the carcinogen in the diet were kept in the Department of Nutrition at Michigan State University and were shipped after autopsy to our laboratory. Because of the long exposure, the total amount of cycad meal or cycasin consumed by these animals was not recorded (groups I to VIII of Table 1).

The rats exposed for short periods (groups IX to XV) were housed at NIH. Food intake was measured daily and the total amount of cycasin consumed was available for each rat, 30 days old at the start of the experiment and for the rats fed for 2 days only (group XV). Attempts to measure the food intake in the rats started at 90 days were abandoned because of spillage of food by the majority. The rats were housed individually and had free access to tap water at all times.

Methylazoxymethanol, the aglycone prepared from cycasin by Dr. MATSUMOTO, was injected at 3 dose levels intraperitoneally into 6 female Fischer rats averaging 210 gm in body weight (Table 2). The tissues obtained at autopsy were forwarded for pathologic studies to our laboratory.

At autopsy, the tissues were fixed in buffered (pH 7.0) 10 per cent formalin. Selected areas were prepared for histologic examination by routine dehydration and paraffin-embedding procedures. Special staining techniques included WILDER's reticulum stain, the periodic acid leukofuchsain reaction with diastase controls for glycogen and mucus supplemented by the mucicarmine stain, the Weigert-van Gieson and the ferrocyanide reaction of Perls (methods outlined by LILIE).

Results

The results of the various experiments with crude cycad meal and cycasin are summarized in Table 1 with respect to the occurrence of intestinal tumors. These neoplasms ranked in relative frequency next to tumors of the liver and kidneys

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1 Cycasin used in this study was obtained through the courtesy of Drs. KURLAND and WHITING of NINDS, NIH.

2 Dr. OLAF MICKELSEN, Department of Nutrition, Michigan State University, generously provided the rats after long-term exposure to cycad meal and cycasin, and Dr. HIROMU MATSUMOTO the rats injected intraperitoneally with the aglycone for inclusion in this study.